CLAIMS

What is claimed is:

1. A method of enriching NK cells, comprising:
   isolating from whole blood or cord blood a mixture of mononuclear cells, wherein the step of isolating the mononuclear cells is performed using density gradient centrifugation, and contacting the mixture of the mononuclear cells for 2-3 days with an anti-CD16 antibody, wherein the anti-CD16 antibody is present at a concentration of 1 mcg/ml, and N-803, wherein the N-803 is present at a concentration of between 0.4-0.8 nM, to activate NK cells; and
   sequentially feeding the activated NK cells with a medium containing N-803 for 15-21 days until the activated NK cells constitute at least 80% of all live cells or until the activated NK cells are enriched to an at least 80-fold expansion, wherein the medium comprises N-803 and human serum.

2. The method of claim 1 wherein the mixture of mononuclear cells comprises T cells, NK cells, NKT cells, and DN T cells.

3. The method of any one of the preceding claims wherein the anti-CD16 antibody is a monoclonal antibody with specificity to human CD16.

4. The method of any one of the preceding claims wherein the mixture of the mononuclear cells contains about 100-500 x 10^6 cells.

5. The method of any one of the preceding claims wherein the step of contacting the mixture is performed in a volume of between about 100-300ml or at a cell density of about 1 x 10^6 cells/ml.

6. The method of any one of the preceding claims wherein the step of contacting the mixture further includes contacting the mixture of the mononuclear cells with an anti-CD3 antibody.

7. The method of claim 6 wherein the anti-CD3 antibody is present at a concentration of between 0.1-1.0 ng/ml.

8. The method of any one of the preceding claims wherein the medium containing N-803 comprises human AB serum.

9. The method of any one of the preceding claims wherein the medium containing N-803 comprises NK MACS medium.

10. The method of any one of the preceding claims wherein the step of sequentially feeding is performed about every 72 hours.

11. The method of any one of the preceding claims wherein the step of sequentially feeding is performed until a total cell number of about 0.5- 5.0 x 10^9 cells is reached.

12. The method of any one of the preceding claims wherein the step of sequentially feeding the activated NK cells is performed in single container.

13. The method of claim 12 wherein the step of contacting the mixture of the mononuclear cells is performed in the single container.

14. The method of any one of the preceding claims wherein the step of sequentially feeding the activated NK cells is performed until NK cells are enriched to an at least 100-fold expansion.

15. The method of any one of the preceding claims wherein the step of sequentially feeding the activated NK cells is performed until NK cells constitute at least 90% of all live cells.

16. A method of expanding NK cells from a mixture of mononuclear cells, wherein the mononuclear cells are isolated from whole blood or cord blood using density gradient centrifugation, the method comprising:

    providing a mixture of the mononuclear cells that contains equal or less than 5% NK cells;

    contacting the mixture of the mononuclear cells for 2-3 days with an anti-CD16 antibody and N-803 to activate NK cells, wherein the anti-CD16 antibody is present at a concentration of 1 mcg/ml, and wherein the N-803 is present at a concentration of between 0.4-0.8 nM; and

    feeding the activated NK cells with a medium containing N-803 for 15-21 days until the activated NK cells constitute at least 80% of all live cells or until the activated NK cells are enriched to an at least 80-fold expansion, wherein the medium comprises N-803 and human serum.

17. The method of claim 16 wherein the mixture of the mononuclear cells is to be obtained from an MHC-matched allogenic source relative to an individual that receives the NK cells.

18. The method of claim 16 or 17 wherein the mixture of the mononuclear cells contains equal or less than 3% NK cells.

19. The method of any one of claims 16-18 wherein the mixture of the mononuclear cells further comprises T cells, NKT cells, and DN cells.

20. The method of any one of claims 16-19 wherein the anti-CD16 antibody is a monoclonal antibody with specificity to human CD16.

21. The method of any one of claims 16-20 wherein the step of contacting the mixture further includes contacting the mixture of the mononuclear cells with an anti-CD3 antibody.

22. The method of claim 21 wherein the anti-CD3 antibody is present at a concentration of between 0.1-1.0 ng/ml.

23. The method of any one of claims 16-22 wherein the medium containing N-803 comprises human AB serum.

24. The method of any one of claims 16-23 wherein the step of feeding comprises sequentially feeding at an interval of about every 72 hours.

25. The method of any one of claims 16-24 wherein the step of feeding is performed until a total cell number of about 0.5- 5.0 x 10^9 cells is reached.

26. The method of any one of claims 16-25 wherein the step of feeding the activated NK cells is performed until NK cells are enriched to an at least 100-fold expansion.

27. The method of any one of claims 16-26 wherein the step of feeding the activated NK cells is performed in an automated manner.

28. The method of any one of claims 16-26 wherein the step of feeding the activated NK cells is performed in a single container.

29. A method of expanding NK cells in an automated bioreactor, comprising:

incubating a mixture of mononuclear cells in an activation medium containing N-803 and an anti-CD16 antibody for 2-3 days, wherein the mononuclear cells are

isolated from whole blood or cord blood using density gradient centrifugation; wherein the anti-CD16 antibody is present at a concentration of 1 mcg/ml, and wherein the N-803 is present at a concentration of between 0.4-0.8 nM;

wherein the mixture of mononuclear cells is contained in a cell culture container while incubating the mixture;

measuring growth of the cells while the cells are in the container;

automatically feeding the cells using a medium containing N-803 for 15-21 days until the activated NK cells constitute at least 80% of all live cells or until the activated NK cells are enriched to an at least 80-fold expansion, wherein the feeding is every 3-4 days, wherein the medium comprises N-803 and human serum;

terminating feeding the cells wherein the terminating is controlled by a result from the step of measuring growth of the cells, wherein the cells are fed until a total cell number of about 0.5- 5.0 x 10^9 cells is reached.

30. The method of claim 29 wherein the container has a volume of between about 200 ml and about 2,500 ml.

31. The method of claim 29 or 30 wherein the step of measuring growth of the cells is performed through a wall of the container.

32. The method of any one of claims 29-31 wherein the step of measuring growth of the cells uses an optical measurement.

33. The method of any one of claims 29-32 wherein the cells are fed until NK cells are enriched to an at least 100-fold expansion.